Transplantation Immunology

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The practice of clinical and experimental transplantation continues to evolve at a rapid pace. To appreciate the current transplant practices, it is first necessary to review transplant immunology in its proper context, ie, as a component of the complex series of events that promote the repair of damaged tissues. These processes are generally catagorized as inflammation, immunity, and tissue repair/reinforcement. In general, there are 3 forms of graft rejection: hyperacute, acute, and chronic rejection. All 3 forms of graft rejection represent pathologic consequences of one or more of these repair-related processes. The various graft rejection responses also illustrate several complex immunologic principles that need to be considered. These include the definition of an alloantigen, the structure and function of major histocompatibility complex molecules, and the behavior of antigen-presenting cells and alloreactive T cells. This review combines these concepts and principles into a discussion of the 3 forms of graft rejection, each of which is addressed at the level of histopathology, pathobiology, incidence, and clinical strategies.

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TRANSPLANTATION immunology has coevolved along with the clinical practice of organ transplantation and represents an interesting blend of traditional immunology and clinical observation. The traditional paradigms of transplant immunology focus on the behavior of T cells in response to graft alloantigen.[1-4] However, recent developments in clinical and experimental transplantation have necessitated both a rethinking of these traditional paradigms[5-7] and an emerging appreciation for the contributions of basic inflammatory mechanisms to graft rejection.[8,9] There are several good reviews on the key immunologic processes that operate in grafts.[1,10,11] In this overview, we will discuss transplant immunology in the broad context of general inflammation, describe the immunologic principles that involve transplantation biology, and relate this information to the practical aspects of clinical transplantation. For this review, we have cited peer-reviewed publications, selected reviews, and book chapters that provide important information relating to clinical transplantation, inflammation, and transplantation immunology. To avoid confusion, we have focused on solid-organ transplantation and deal separately with bone marrow transplantation (BMT).

ALLOIMMUNITY AS AN INFLAMMATORY RESPONSE

To understand the various mechanisms by which allografts are rejected, it is necessary to appreciate the pattern of biologic events that occur in response to tissue damage. In general, the responses to tissue damage from transplantation surgery range from immediate damage control measures through immune surveillance for invasion by opportunistic pathogens and, if necessary, pathogen eradication. In patients who are serologically presensitized to alloantigens (ie, graft antigens recognized as "nonself"), this can rapidly proceed to a pathologic thrombotic response (hyperacute rejection). When graft alloantigens are encountered by T cells, the inflammatory responses intensify, and pathologic tissue destruction ensues (acute rejection). When these T-cell–dependent responses to graft alloantigens are controlled by immunosuppressive drugs, acute rejection is avoided, but tissue repair mechanisms are allowed to engage. If the repair and structural reinforcement process is prolonged, pathologic tissue remodeling occurs (chronic rejection). The characteristics of each type of rejection are summarized in the Table. As will be described below, each form of graft rejection represents a pathologic (relative to the graft) manifestation of one or more of these normal biologic processes. Thus, it is appropriate to briefly review the biologic events initiated by tissue damage.

In general, there are antigen-independent causes of tissue damage associated with allograft transplantation, including peritransplant ischemia, mechanical trauma, and reperfusion injury.[12,13] There are also antigen-dependent causes, such as immune-mediated damage to graft tissues. Any tissue damage in the graft compromises graft acceptance by promoting graft inflammation, a complicated array of events that promotes control, pathogen surveillance/eradication, and tissue repair. These 3 processes can each contribute in unique ways to the demise of the allograft, and the clinical control of allograft rejection will require the control of each of these necessary but potentially harmful processes.

Damaged tissues release proinflammatory mediators, such as Hageman factor (factor XII), that trigger several biochemical cascades designed primarily for immediate damage control. These include the clotting cascade, which induces fibrin to restore vascular integrity, and several related fibrinopeptides that initiate tissue surveillance by promoting local vascular permeability and by attracting neutrophils and macrophages with subsequent cytokine production.[14,15] This process is assisted by the principal product of the kinin cascade, bradykinin, which promotes vasodilatation, smooth muscle contraction, in-

Summary of Allograft Rejection Responses*

Features	Hyperacute Rejection	Acute Rejection	Chronic Rejection
Incidence	Rare (1%, due to rigorous pretransplant testing)	Common (50%), depending on therapeutic strategy	Common (50%), increases with increasing episodes of acute rejection
Time of onset	Minutes to days after transplantation	Usually within 1 y of transplantation	Usually within 5-10 y, but can be as early as weeks after transplantation
Histopathology	Thrombosis, platelet and PMN accumulation, hemorrhage	Escalating leukocytic infiltration, edema, tissue necrosis	Leukocytic infiltration may be present, interstitial fibrosis, neointima formation in arteries and veins
Pathobiology	Endothelial cell damage, vascular thrombosis, tissue infarction	T-cell activation by graft alloantigens, progressive, destructive tissue infiltration	Prolonged vascular stimulation, prolonged liberation of cytokines, chemokines, and tissue growth factors; pathologic vascular and tissue remodeling; overproduction of extracellular matrix by smooth muscle cells and fibroblasts
Primary mediators	Alloantibodies, complement	T cells (alloantibodies?)	Alloantibodies, T-cell products, tissue growth factors
Current therapeutic strategies	None	Azathioprine, steroids, cyclosporine, tacrolimus, OKT3, mycophenolate mofetil	None
Experimental therapeutic strategies	Antibody removal by column adsorption or plasmapheresis	Anti–IL-2 receptor, anti-CD4 monoclonal antibody, rapamycin, monoclonal antibodies to leukocyte adhesion molecules	Angiopeptin, lefludamide, rapamycin, mycophenolate mofetil

*PMN indicates polymorphonuclear leukocyte; OKT3, anti-CD3 monoclonal antibody; and IL-2, interleukin 2.

creased vascular permeability, and a local warning signal, ie, pain.[12,13,16]

Macrophages dominate the early phase of tissue surveillance, during which time they may encounter evidence of invasion by pathogens, such as bacterial cell wall products. In addition, antibodies to many common pathogens are present in the circulation and can enter the damage site due to the increased vascular permeability, where they form antigen-antibody complexes. These complexes not only target pathogens for phagocytosis, but also stimulate macrophages to secrete pyrogenic, proinflammatory cytokines like interleukin 1 (IL-1). In addition, immune complexes activate the classical complement cascade, which in turn releases bioactive intermediates like C3a and C5a. These contribute to local inflammation in a variety of ways, acting as powerful chemoattractants and initiating mast cell degranulation. Mast cells provide the damage site with histamine and 5-hydroxytryptamine, which increase vascular permeability; with prostaglandin E_2, which promotes vasodilatation and vascular permeability; and with leukotrienes B_4 and D_2, which promote leukocyte accumulation and vascular permeability. Overall, these processes result in the cardinal signs of inflammation: swelling due to edema, redness due to vasodilation, fever due to cytokine release, and pain. As will be discussed below, hyperacute allograft rejection and xenograft rejection are related graft pathologies promoted by antibody-associated elements of these early proinflammatory responses.[13,16]

One interesting manifestation of these early proinflammatory responses that can occur in vascularized grafts is reperfusion injury.[12] While the organ is in transit between the donor and recipient, it is often kept on ice. This temporary interruption of vascular perfusion, which can last up to 36 hours, allows bioactive proinflammatory agents to accumulate in the graft. When the vascular flow is restored during transplantation, free radicals, cytokines, and other bioactive proinflammatory agents surge through the graft vasculature, causing widespread inflammation within the graft. When severe, this inflammation can significantly compromise the physiologic function of the graft during the immediate postoperative period.[12,16]

When macrophages encounter evidence of invasion at a damage site, they release cytokines such as tumor necrosis factor (TNF) and IL-1, which significantly heighten the intensity of the local inflammation by stimulating proinflammatory endothelial responses.[15,17] These endothelial changes help to recruit large numbers of T cells to the graft site. These T cells upgrade immune surveillance procedures and initiate pathogen eradication programs, if necessary. Many of these eradication programs are used within allografts when T cells encounter graft alloantigens. Thereafter, the T-cell–dependent campaign to contain and eliminate allogeneic cells, known clinically as acute allograft rejection, is relentless and overwhelming. T-cell responses to alloantigens are well studied, and much of this review will address the mechanisms by which T cells recognize and respond to graft alloantigens during acute graft rejection.

If macrophages enter a damage site and fail to encounter evidence of pathogen invasion, or after invading pathogens have been effectively eliminated by the immune system, the macrophages begin to promote the repair and structural reinforcement of the damaged tissues. This ancient and complex process involves endothelial cells, smooth muscle cells, fibroblasts, and the extracellular matrix, all operating in a coordinate fashion to promote tissue regeneration and neovascularization. The coordination of this process is mediated by complex patterns of biochemical communication.[18] Some of these involve direct contact of cells with each other or with the matrix, often via various integrin adhesion molecules. Others involve indirect contact via soluble mediators, like IL-1 and IL-6 , or tissue growth factors like transforming growth factor, fibroblast growth factor, or platelet-derived growth factor. The net result of these interactions is the restoration of tissue integrity and function. In general, these processes are beneficial, but aberrations can sometimes lead to undesirable, pathologic tissue remodeling. One example of an aberrant response is the vascular remodeling and interstitial fibrosis associated with chronic allograft rejection.

DEFINITIONS AND BASIC IMMUNOLOGIC MECHANISMS OF ALLOANTIGEN RECOGNITION

The remainder of this review will address alloantigen-dependent inflammatory responses. It is worthwhile to define alloantigens, to describe how alloantigens are observed by the immune system, and to delineate the resultant immune responses following alloantigen exposure.

Major Histocompatibility Complex Function

The critical immune components that facilitate the recognition of foreign proteins are the major histocompatibility complex (MHC) class I and class II molecules. The primary function of MHC proteins is to scavenge peptides and exhibit them on the surface of cells for review by T cells. The structure and function of the MHC molecules have been extensively reviewed.[19-21] In general, MHC class I molecules are used to scavenge peptides derived from proteins of

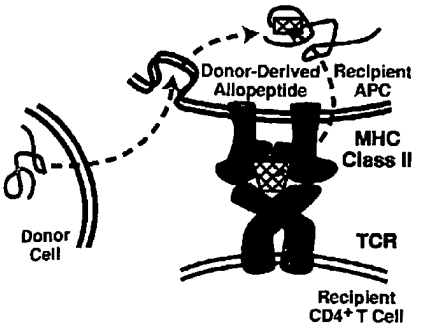

Figure 22-1.—One of the 3 pathways of the presentation of alloantigenic peptides. Proteins derived from graft cells are obtained by recipient antigen-presenting cells (APCs), processed, and displayed via recipient major histocompatibility complex (MHC) class II molecules to recipient CD4+ T cells. Adapted with permission from VanBuskirk et al.[27]

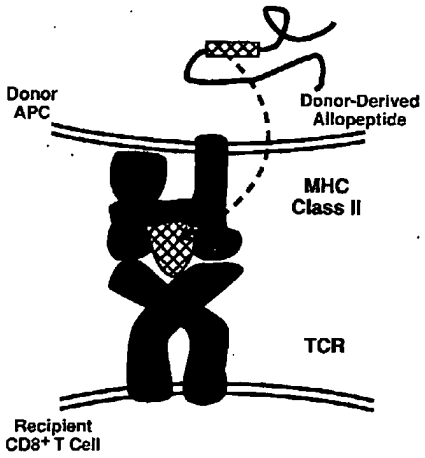

Figure 22-2.—One of the 3 pathways for the presentation of alloantigenic peptides. Proteins produced by graft-derived antigen-presenting cells (APCs) are processed and displayed via graft-associated major histocompatibility complex (MHC) class I molecules to the recipient CD8+ T cells. TCR indicates T-cell receptor. Adapted with permission from VanBuskirk et al.[27]

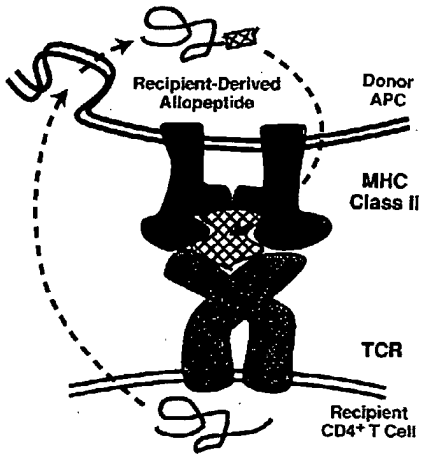

Figure 22-3.—One of the 3 pathways for the presentation of alloantigenic peptides. Proteins derived from the graft recipient are obtained by graft-derived antigen-presenting cells (APCs), processed, and displayed by graft-associated major histocompatibility complex (MHC) class II molecules to recipient CD4+ T cells. TCR indicates T-cell receptor. Adapted with permission from VanBuskirk et al.[27]

the internal cellular environment, while MHC class II molecules are used to scavenge peptides derived from the external environment. T cells use their T-cell receptors to recognize and bind to MHC molecules for the purpose of reviewing these bound peptides.[22]

What Constitutes an Alloantigen

Traditionally, an alloantigen is a foreign peptide derived from polymorphic regions of allelic variant proteins found in different members of the same species. These peptides are bound to self–MHC molecules for display to self–T cells during "self–MHC-restricted" T-cell alloactivation. Individuals are not tolerant to self-proteins, but only to specific peptides of self-proteins, specifically those peptides that can bind to their particular MHC molecules.[23-25] T cells that recognize these self-peptides held by self–MHC molecules are usually deleted in the thymus, resulting in tolerance to the peptides, and thus the parent protein. By default, the residual T cells can identify peptides that are non-self and presumably derived from foreign proteins. During immune responses to most environmental antigens, only self–MHC molecules are available. However, immune responses to allogeneic cells are much more complicated: in the unusual case of allografts, cells bearing allogeneic MHC molecules are also present. These allogeneic MHC molecules are quite capable of presenting peptides to self–T cells. The problem arises because each MHC molecule has a unique peptide-binding motif, ie, pattern of amino acids that permit MHC binding.[23,26] In general, allogeneic MHC molecules bind different peptides than self-MHC. This allows them to bind some of the peptides from self-proteins that were not used in the developmental, self-tolerization process. These cryptic self-peptides become operationally foreign and can serve as alloantigens during allo-MHC–restricted T-cell activation. Thus, an alloantigen can be (1) graft-derived peptides bound by self–MHC molecules; (2) graft-derived peptides bound by graft MHC molecules; or (3) self-peptides bound by graft MHC molecules (Figures 22-1–22-3).[27] The immunologic consequence of these multiple types of alloantigens is a significant increase in the frequency of T cells recruited into the allogeneic response. As many as 1 in 2000 T cells can respond to allogeneic cells, compared with about 1 in 100 000 to 1 in 1 million T cells that can respond to a nominal antigen, such as tetanus toxoid.[27] This explains why primary T-cell responses to allogeneic cells are so much more intense than primary T-cell responses to most environmental antigens.

What Constitutes an Antigen-Presenting Cell

Most cell types express MHC class I molecules. Some also express MHC class II molecules. Thus, most cell types, theoretically, should be able to present alloantigens to T cells. However, productive peptide presentation is accomplished only by a few specialized antigen-presenting cells (APCs). These APCs include dendritic cells, macrophages, B cells, and endothelial cells.[28] The distinguishing feature of these cells is their unique display of costimulatory adhesion molecules. These adhesion molecules serve as ligands for counterreceptors on T cells. Several costimulatory receptor/ligand systems have been identified, including CD28/B7, CD2/LFA-3, VLA-4/VCAM-1, and LFA-1/ICAM-1. There appears to be a general hierarchy of APCs (dendritic cells > macrophages > B cells > endothelial cells > fibroblasts), probably based on the panorama of costimulator systems displayed by each cell type.[29-31] Other factors that influence APC function are the immune status of responding T cells (naive vs memory) and proinflammatory mediators that may be present at the site of APC–T-cell contact.[32-34] Since allografts contain APCs and are sites of intense inflammation that attract additional APCs from the host, immune responses at graft sites are complex and powerful.

What Happens When Alloantigen Is Presented by a Competent APC to a T Cell

When T cells encounter antigenic peptides displayed by competent APCs, they are activated to produce lymphokines, perhaps to acquire cytolytic activity, and ultimately to proliferate. In general, CD4+ T cells survey peptides displayed by MHC class II molecules, ie, monitor proteins in the extracellular environment, while CD8+ T cells survey peptides displayed by MHC class I molecules, ie, monitor proteins from the intracellular environment.[10] Proinflammatory cytokines, such as IL-4 and interferon γ (IFN-γ), that may be present in the environment can influence the result of T-cell activation, including the pattern of cytokines that are secreted, and the acquisition of cytolytic activity. In turn, this defines the character of the subsequent immune response.[35]

Various hypotheses have been formulated to explain how each of the different

T-cell functions contributes to allograft rejection. Cytotoxic T-lymphocyte (CTL) activity has long been postulated to be the primary mediator of graft rejection, given that graft-reactive CTLs are frequently found in rejecting allografts.[2] However, there is compelling evidence that rejection can occur in the absence of CTLs.[5,6] In this situation, it has been postulated that lymphokines promote acute rejection in a delayed-type hypersensitivity (DTH) fashion.[1] This is an attractive model, as both acute rejection and DTH responses are in vivo inflammatory responses driven by local deposition of antigen and characterized by cellular infiltration, edema, and, in extreme cases, tissue necrosis. In this regard, the pattern of lymphokines secreted by alloactivated T cells could be critical. It has been postulated that T-cell activation in the presence of IFN-γ activates cytolytic T_H1 cells causing acute rejection, whereas T cells activated in the presence of IL-4 activate noncytolytic T_H2 cells that permit graft acceptance.[36] However, there remains considerable controversy regarding this issue.[7,37]

The source of the APCs involved in the allogeneic response may also influence allograft survival. Allorestricted T-cell responses (those stimulated by graft APCs) have been implicated as the prime mediators of acute graft rejection. Self-restricted T-cell responses (those stimulated by self-APC) may contribute to rejection indirectly, for example, by interaction with self–B cells to promote alloantibody production. As will be discussed below, alloantibodies are deleterious to graft function in many ways. Self-restricted T cells may also interact with graft-infiltrating macrophages, which comprise about half of the graft infiltrate, to produce local DTH-like responses that may compromise graft function.[38]

TYPES OF GRAFT REJECTION

Hyperacute Rejection

Histopathology.—Hyperacute rejection is characterized histologically by the presence of large numbers of polymorphonuclear leukocytes (PMNs) within the graft vasculature in association with widespread microthrombi formation and platelet accumulation.[39,40] Vascular integrity may be compromised, as evidenced by hemorrhage. In general, there is little or no interstitial leukocytic infiltration.

Pathobiology.—Hyperacute rejection usually occurs within minutes to hours of graft implantation. This form of rejection is dependent on the presence within the graft recipient of preformed circulating antibodies with specificities for foreign protein polymorphisms displayed by the graft endothelia. Chief among these are the ABO blood group proteins and the MHC-encoded proteins. Anti-MHC alloantibodies can result from prior transfusions, prior graft rejection, or prior pregnancy. Immediately on the reestablishment of blood flow to the graft, the circulating antibodies bind to the graft endothelia and initiate the complement cascade, leading to platelet activation and neutrophil accumulation and endothelial damage.[40,41] Endothelial cell damage or destruction exposes the subendothelial matrix, a potent stimulus for the coagulation cascade. Endothelial cells are equipped to avoid complement-mediated destruction. However, the bound alloantibodies or cascade products can activate endothelial cells, causing them to shift from an anticoagulant to a procoagulant functional status. In general, this leads to rapid thrombosis, loss of vascular integrity, infarction, and loss of graft physiologic function.

Incidence.—Hyperacute rejection was a common problem in the early days of clinical transplantation. It has become relatively rare (fewer than 1% of transplants) since the introduction of routine pretransplant screening of graft recipients for antidonor alloantibodies.[42-44]

It should be noted that xenografts are also hyperacutely rejected, because of the presence of circulating, "natural" xenoantibodies in all mammalian species.[41,45] The potential use of xenografts to alleviate the organ donor shortage has stimulated intense experimental interest in the mechanisms and management of hyperacute rejection.[39,40]

Clinical Strategies.—Avoidance of high-risk donor-recipient combinations remains the only effective clinical strategy for this form of graft rejection. To date there is no effective pharmacologic intervention that can control hyperacute rejection. Fortunately, hyperacute rejection has been virtually eliminated by the early development and continuing refinement of rapid, sensitive testing methods for detecting donor-reactive antibodies. These methods are collectively referred to as pretransplant crossmatch tests.[46] In addition to the conventional complement-dependent cell cytotoxicity method, cross-match testing can now be done by flow cytometric or enzyme-linked immunosorbent assay (ELISA) methods. The advantages and disadvantages of these newer crossmatch methods are currently being evaluated. In addition, several pharmacologic agents that can interfere with the complement or clotting cascades are currently being evaluated for their abilities to interfere with the development of hyperacute rejection.

Acute Rejection

Histopathology.—Acute rejection is characterized by the presence of leukocytes, dominated by equivalent numbers of macrophages and T cells within the graft interstitium.[1,2] In the absence of concurrent infection, few PMNs are found. Early acute rejection displays only a few focal regions of perivascular infiltration, which can progress to large widespread pools of leukocytes that disrupt the tissue architecture. Leukocytic infiltration of vascular tissues is associated with an especially poor prognosis. In later stages of rejection, there is also microscopic evidence of tissue destruction, including pyknotic nuclei and cellular debris.

Pathobiology.—Within the interstitial infiltrate of acutely rejecting grafts is a small number of recipient-derived, donor alloantigen–reactive T cells, on which the process of acute rejection is absolutely dependent.[2] Infiltrating graft-reactive T cells are very rare and usually constitute less than 0.5% of the leukocytic infiltrate. These T cells are activated by contact with foreign, graft-related peptides that must be displayed to them by specialized APCs (ie, macrophages and dendritic cells) of either graft or donor origin.[10] However, the exact mechanisms by which these alloantigen-activated T cells promote graft destruction remain ill-defined. The conventional model suggests that donor-specific cytolytic T cells become activated and destroy graft cells by lethal direct contact.[2] While this may occur, it is not essential for the rejection process.[5,6] Instead, the critical destructive activities of graft-reactive T cells are probably promoted by cytokines. All activated T cells, including cytolytic T cells, secrete a variety of proinflammatory cytokines.[1] One of these, TNF, is directly cytotoxic. Others, like IFN-γ and IL-1, activate the cytotoxic activities of other infiltrating cell types, especially macrophages. Several lymphokines, like IFN-γ, TNF, IL-1, and IL-4, are vasoactive and promote proinflammatory microvascular functions related to leukocytic infiltration.[17,47] These are generally incompatible with the physiologic function of the microvasculature, ie, regulated tissue perfusion for nutrient supply and waste disposal. As a consequence of these T-cell–regulated inflammatory responses, graft infiltration becomes aggressive and relentless, leading to tissue distortion, vascular insufficiency, and cell destruction. Within a few days, these processes can compromise graft function beyond repair.

There is a related form of graft rejection, called *accelerated acute rejection*, that occurs in individuals who have sen-

sitized T cells that were generated during the rejection of a previous graft and who reencounter those same alloantigens on a second graft. In these individuals, rejection occurs very rapidly via mechanisms associated with leukocytic infiltration and tissue destruction. Usually, this form of rejection is too intense to be controlled by routine antirejection therapy. This consequence of previous T-cell allosensitization, in conjunction with the immunologic consequences of alloantibodies produced after acute rejection and the well-established immunologic cross-reactivity among alloantigens, probably account for the relatively poor graft survival in retransplant patients compared with primary graft recipients.[43]

Incidence.—The history of increasingly successful clinical transplantation correlates directly with the development of increasingly effective immunosuppressive strategies to avoid or control acute rejection. When generally accepted, cyclosporine-based immunosuppressive strategies are used, acute rejection episodes occur in approximately 50% of transplant patients. However, immediate graft loss due to acute rejection occurs in fewer than 10% of these patients because of the development of effective antirejection therapies, such as treatment with OKT3, an immunosuppressive monoclonal antibody directed at the CD3 component of the human T-cell receptor.

Clinical Strategies.—To date, the control of acute rejection has been the primary aim of pharmacologic intervention in transplantation. The drugs used for this purpose generally target critical intercellular signaling events of T cells that are necessary for their functional activation. Thus, these drugs can inhibit clonal expansion (eg, azathioprine) and cytokine production (eg, steroids and cyclosporine). The best therapeutic strategies use combinations of several agents. This optimizes the immunosuppressive effects at minimal drug dose and therefore minimizes adverse effects.[48,49] Several commonly used agents are azathioprine, a purine analog, blocks DNA replication and RNA transcription; steroids are cytotoxic for T cells and inhibit cytokine production by mononuclear cells; and both polyclonal and monoclonal antilymphocyte antibodies are used to temporarily deplete circulating T lymphocytes to provide a period of immunologic unresponsiveness. Cyclosporine and tacrolimus are low-molecular-weight fungal metabolites used to inhibit lymphokine production by T cells. The advent of cyclosporine revolutionized clinical transplantation. However, each of these agents has adverse effects (eg, bone marrow suppression by azathioprine, osteoporosis and adrenal suppression with steroids, and renal toxicity with cyclosporine and tacrolimus). Also, generalized immunosuppression is associated with increased incidence of infection and malignancy in transplant patients.

Recently, a variety of new immunosuppressive agents have been developed that inhibit acute rejection and, in some cases, alloantibody production as well. These include mycophenolate mofetil, which is in clinical use, and rapamycin and anti–IL-2 receptor monoclonal antibody, which are in clinical trials.[50-52] Recent studies using mycophenolate mofetil in combination with cyclosporine-based immunotherapy indicate a much lower incidence of acute rejection compared with traditional cyclosporine-based therapies.[53] Each of these new agents exhibits a unique spectrum of actions that distinguishes them from cyclosporine and offers attractive alternatives for transplant physicians. The clinical challenge will be to integrate these drugs into new, effective therapeutic strategies that use their advantages and minimize negative interactions or toxic effects.

Chronic Rejection

Histopathology.—This form of rejection is characterized by the development of blood vessel luminal occlusion due to progressive neointimal formation within the large to medium arteries and, to a lesser extent, veins of the graft.[54] This neointima contains, among other things, smooth muscle cells and large amounts of extracellular matrix material. Vascular lesions are confined to the graft, and the graft vessels appear to be affected at random and to varying degrees, even within the same region of the graft.[55] The vascular lesions differ somewhat from those associated with atherosclerosis, but are similar to lesions that sometimes develop after balloon catheterization or vein grafting. The formation of vascular lesions is frequently associated with prominent interstitial fibrosis, reflecting the ongoing deposition of mature connective tissue in the interstitium.[54,55] Associated leukocytic infiltration is usually mild or even absent. In later stages, there is severe tissue distortion, vascular insufficiency, atrophy of graft parenchymal tissues, and, terminally, the irreversible loss of graft function. While the development of these pathologic processes can take years, certain physiologic and immunologic factors can accelerate their development to within a few weeks of transplantation.[54,56,57]

Pathobiology.—Chronic rejection is not simply delayed acute rejection. Rather, it represents a pathologic tissue-remodeling response that develops at a variable rate as a consequence of peritransplant and posttransplant vascular trauma.[9,54,56-58] Graft vascular endothelia that are traumatized by mechanical, ischemic, immunologic, or pharmacologic injury produce cytokines and tissue growth factors that initiate vascular repair and reinforcement mechanisms. Vascular smooth muscle cells respond to these signals by changing from a contractile to a synthetic phenotype, proliferating, migrating toward the endothelial cells, and producing large amounts of new matrix material. When these events occur in the subendothelial region of large vessels, an increasingly occlusive neointima is formed. Interstitial fibroblasts may also respond to some of these endothelial signals by producing collagen, which would account for the concurrent development of interstitial fibrosis. Additionally, the reduced blood flow through occluded vessels may contribute to regional tissue ischemia, death, and fibrosis.

These tissue-remodeling responses may be acutely stimulated soon after transplantation, primarily as a response to peritransplant ischemia and reperfusion injury. They may be stimulated chronically by the use of vasoactive immunosuppressants like cyclosporine or by physiologic conditions leading to vascular stress like high blood pressure or hyperlipidemia.[59-62] One strong stimulus to their development is the onset of posttransplant allosensitization, evident as an acute rejection episode, the production of donor-reactive alloantibodies, or both.[58,63,64] Many clinical studies show that posttransplant alloantibodies pose a clear risk for reduced graft survival.[65,66] Studies with animals have implicated alloantibodies as a cause of graft neointimal formation, presumably by antagonism of graft endothelia.[67] However, alloantibodies are not essential for neointimal formation, and some cytokines associated with acute rejection, like IFN-γ, have also been associated with neointimal formation.[58] In general, it appears that some degree of chronic graft remodeling may be unavoidable in graft recipients and that the rate of the remodeling process can be influenced by several factors, particularly posttransplant allosensitization.

Incidence.—Measurement of the incidence of chronic rejection depends on the time frame selected. Currently, 10-year graft survival can be reliably evaluated, and it appears that by this time, chronic graft rejection occurs in about 50% of transplant patients, as determined by evidence of graft dysfunction and transplant renal biopsy.[58] When analyzed in more detail, it becomes apparent that the patient subpopulation with chronic rejection is generally the same

patient subpopulation with previous acute rejection episodes.[68,69] This illustrates the immunologic relationship between acute rejection, which is primarily a pathologic inflammatory response, and chronic rejection, which is primarily a pathologic tissue-remodeling response. It also suggests that the incidence of chronic rejection may fall substantially in patients treated with new immunosuppressive agents that reduce the incidence of acute rejection episodes, posttransplant alloantibody production, or both.

Clinical Strategies.—Currently, there is no accepted therapeutic strategy for the treatment of chronic rejection. Indeed, by the time chronic rejection is manifest clinically, it may be irreversible. Consequently, prophylactic strategies will have to be developed. These should be aimed at the risk factors for chronic rejection. For example, strategies should be developed that minimize peritransplant ischemia and reperfusion injury. These would include the wider use of pulsatile graft perfusion during transport and peritransplant graft treatment with pharmacologic agents that interfere with physiologic and immunologic mediators of vascular trauma.[9] In addition, it is essential to develop strategies that completely avoid the development of acute rejection (allosensitization). Reliance on antirejection therapies to reverse acute rejection is not sufficient in this regard. Finally, therapeutic strategies that interfere directly with fundamental mechanisms of tissue remodeling should be developed. Current immunosuppressants, which were developed to interfere with immunologic mechanisms of graft recognition, have little effect on tissue-remodeling mechanisms. One promising approach involves angiopeptin, a somatostatin analog under clinical trial, which appears to interfere with smooth muscle cell participation in the development of chronic vascular lesions.[70]

BONE MARROW TRANSPLANTATION

Bone marrow transplantation is the preferred treatment for many hematologic malignancies, including (1) diseases of hematopoietic origin such as leukemias, lymphomas, and aplastic anemias; (2) rescue therapy following radiation and chemotherapeutic treatment for malignancy; (3) genetic disorders such as severe combined immunodeficiency, thalassemia, or sickle cell anemia; and (4) therapy for genetic deficiencies.[71] Because the patient's immune system is ablated prior to transplantation, the greatest risk after engraftment is not rejection of the bone marrow by the patient's immune system, but rather an immune attack on host cells by the transplanted bone marrow (graft-vs-host disease [GVHD]). In addition to GVHD and recurrence of the original disease, patients are at risk for rejection of the bone marrow graft, organ damage resulting from the preparative chemotherapeutic and radiation treatments, and infection. Nonetheless, clinical use of BMT burgeoned in recent years, fostered by the ever-increasing survival of recipients, the direct result of improvements in histocompatibility analysis and more effective methods of treating GVHD and viral infections that plagued earlier transplant patients. Bone marrow or stem cells can be obtained from a variety of sources, including autologous donation, donation by MHC-matched siblings or relatives, MHC-matched unrelated donors, and cord blood–derived stem cells. At present all donors are matched to recipients for MHC class I and class II (*HLA-A*, *HLA-B*, and *HLA-DR* loci). The development of rapid DNA-based typing methods for HLA analysis has greatly improved matching for *DR* loci and will soon be used for HLA-A and HLA-B typing as well.[44] Despite these efforts, however, GVHD remains the major barrier to BMT and stem cell transplantation. Ironically, the occurrence of GVHD is not entirely detrimental, since patients who experience some GVHD have a lower incidence of leukemia recurrence following BMT.

Currently, there is an enormous effort to develop worldwide bone marrow and cord blood registries to increase the identification of MHC-matched BMT donors. The National Marrow Donor Program in the United States currently has approximately 2 million registered donors. This effort has been hampered by the limited number of potential donors and the high degree of genotypic diversity in some racial groups. These limitations make it increasingly important to develop therapeutic strategies that permit MHC-mismatched BMT. One promising therapeutic strategy is to transduce donor T cells with a suicide gene prior to transplantation. Two independent groups[22,72] have reported the inhibition of GVHD by transducing donor T cells with viral thymidine kinase using either the IL-2 promoter or a retroviral vector, and subsequently treating with ganciclovir. Indeed, Bonini and colleagues[72] have successfully controlled GVHD in 5 patients by ganciclovir-induced elimination of the transduced donor cells.

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